Development of a selective TOCSY experiment and its use in analysis of a mixture of related compounds

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A selective TOCSY experiment based on the recently described DPFGSE selective excitation sequence has been developed: it employs gradient purging pulses which result in excellent lineshapes free of antiphase dispersive components, and has proven useful in the identification of a number of impurities in a pharmaceutical intermediate.

Recently, Stott *et al.* have described a new method of selective excitation called 'double pulsed field gradient spin echo' (DPFGSE).[1,2] The method gives exceptionally clean selective excitation. They have used this selective excitation scheme to develop a one dimensional NOE experiment, the DPFGSE-NOE, which gives excellent quality NOE spectra devoid of the subtraction artefacts normally associated with the steady state NOE difference experiment.

Using this excitation scheme, we have developed a selective TOCSY experiment.[3–5] DANTE pulses have been used instead of shaped pulses within the DPFGSE sequence [Fig 1(*a*)], due to spectrometer hardware limitations, but the quality of the selective excitation remains high. This approach has the advantage of allowing the bandwidth of the selective excitation to be easily tailored by adjusting the DANTE delay. The TOCSY transfer can be achieved by a period of spin locking following on from the selective excitation. The DIPSI[6,7] sequence was chosen in this case. However, such a sequence can result in severely distorted lineshapes.[8,5] These distortions arise because any magnetisation which is not purely single component, prior to and after the period of spin locking, results in zero quantum coherence which gives an antiphase dispersive contribution to the lineshape. Thus poorer quality spectra are obtained and interpretation is less straightforward in that direct comparison with a proton spectrum or with expected multiplet patterns is not possible. This is clearly illustrated in Fig. 2(*a*), which shows a selective TOCSY spectrum of sucrose following excitation of the anomeric proton. The triplet due to H4 and the double doublet due to H2 are both severely distorted. A z-filter, employing a variable delay list,[9,10] is one way of reducing these undesired effects, but this necessitates long acquisition times as the phase cycle must be completed for each value of the z-filter delay, of which there are typically many. In addition, even with all parameters set up optimally, this method can never remove all of the phase anomalies.[8]

A superior method for removing these zero quantum effects is provided by dephasing in an inhomogenous B_0 field, as described by Davis *et al.*[8] The method is based around a period of spin locking which is executed concurrently with a gradient pulse, before and after the mixing sequence. This method gives exceptionally clean two dimensional TOCSY spectra,[8] and a similar scheme has been used by Dalvit in a 1D TOCSY.[4] The essence of the method is that zero quantum coherence precesses during the gradient pulse. Its offset becomes spatially dependent and it is therefore dephased. The pulse sequence incorporating these purging pulses and the DIPSI mixing sequence is shown in Fig. 1(*b*). Fig. 2(*b*) shows a selective TOCSY spectrum of a sucrose test sample—the lineshape and multiplet structure is greatly improved, and compares well to the normal proton spectrum.

The excellent selectivity of the DPFGSE sequence suggested to us that this TOCSY sequence might prove useful in the analysis of low level impurities in a mixture. This type of analysis could be tackled by LC-NMR,[11] but this technique is not without its problems. Replicating chromatography at the high loading levels required for LC-NMR is not always straightforward, and lower loading levels can lead to inadequate sensitivity. Also, instrument time is a factor, with probe changes

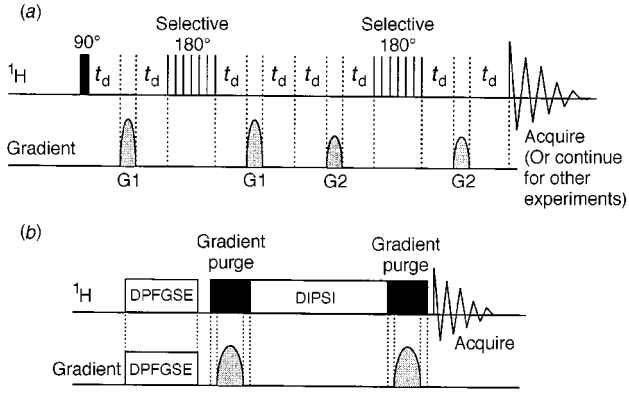

Fig. 1 (*a*) Pulse sequence for the DPFGSE selective excitation. The DANTE pulse consists of 200 π/200 pulses separated by a delay of 125 μs. (*b*) Additional pulses for the selective 1D-TOCSY experiment.

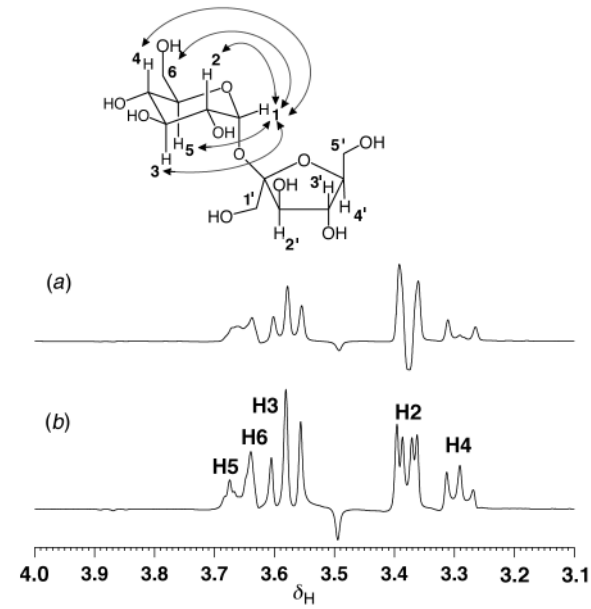

Fig. 2 (*a*) Selective TOCSY spectrum of sucrose obtained using only a period of DIPSI mixing after selective excitation. Phase distortions are apparent. (*b*) Selective TOCSY spectrum obtained using the sequence in Fig. 1(*b*), showing almost complete removal of distortions. The TOCSY mixing time was 100 ms, the purge spin lock 10 ms and the gradient pulse 9.8 ms at approximately 15 G cm^{-1} (a 100 μs delay precedes and follows the gradient).

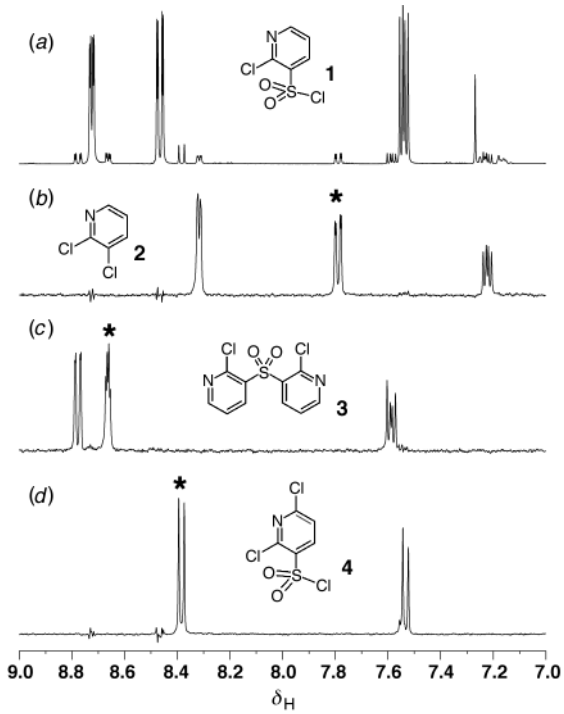

Fig. 3 (*a*) 400 MHz ^{1}H NMR spectrum of **1** in DMSO-d_6. A number of low level impurities are also apparent. (*b*)–(*d*) Selective 1D-TOCSY spectra obtained after selective excitation of the marked peaks. Each spectrum is consistent with the impurities **2**–**4**. Spectra in (*b*)–(*d*) acquired with 64 transients.

significantly increasing the time taken for analysis of a sample. Diffusion ordered spectroscopy[12,13] is perhaps a promising alternative, but this technique requires a significant difference in the diffusion coefficients of the species involved; this may well not be the case for typical impurities of related structure. The selective TOCSY experiment described above might provide another fast approach. The method would require that one impurity peak be resolved from other components. In these circumstances, a partial spectrum of the impurity could be acquired, and in the case of simple compounds consisting of one spin system, an essentially normal proton spectrum could be obtained.

This approach was tested on a sample which contained a number of impurities requiring identification. The major component in the mixture was the sulfonyl chloride **1**, but a number of other peaks were apparent in the spectrum [Fig. 3(*a*)]. Using the selective TOCSY experiment described above, the resonance at 7.8 ppm was excited, resulting in the spectrum shown in Fig. 3(*b*). This spectrum clearly shows the excellent suppression of the main component, together with a lineshape which compares favourably to that of the normal proton spectrum. The spectrum clearly represents a component in which the three aromatic protons are still present, but the relatively large chemical shift changes suggest different substituents to the main component. The spectrum is therefore consistent with that of the suspected impurity **2**. Similarly, selective excitation of the resonance at 8.65 ppm resulted in the spectrum shown in Fig. 3(*c*). As for the first impurity, the spectrum indicates a disubstituted pyridine system, but the similarity of chemical shifts to the main component suggests that these resonances are due to the dimeric sulfone **3**, another suspected impurity. Note that in this spectrum too, lineshape is good and the main component is well suppressed, despite the fact that the selectively excited proton is only 0.1 ppm from a resonance of the main component.

Finally, the resonance at 8.4 ppm was selectively excited. This gave rise to the 1D-TOCSY spectrum shown in Fig. 3(*d*). Clearly, this component exhibits only two proton resonances, indicating a tri-substituted product. It was therefore assigned to the dichloro sulfonyl chloride **4**, which was another suspected contaminant. Note that one of the two resonances in this component was completely overlapped with the main component in the normal proton spectrum. The excellent suppression of the main component, however, means that this peak can clearly be observed with its normal lineshape and doublet structure.

Overall, the three experiments took a total of 15 min to acquire. Subsequently, the sample was analysed by LC-NMR. This took several hours, including time for probe changes and setting up the chromatography. The conclusions reached were the same. Mass spectrometry also confirmed the identity of the three impurities.

The impurities in this sample were present at a level of approximately 4% w/w each. The level of suppression afforded by the selective TOCSY experiment is such that the residual peaks arising from the main component are approximately 2% of the level of each impurity. This figure suggests that the level at which impurities could be detected while retaining a useful degree of suppression of the main peak is rather lower than the 4% in this sample. It is estimated that 0.5% could be regarded as the limit of detection.

Of course, this selective TOCSY experiment will not give a complete, 'normal' spectrum of every component in every mixture; it may be that peaks are not resolved from the main component, or more complicated structures with many spin systems will not allow transfer to all parts of the molecule. However, in the case of many pharmaceutical compounds, we are looking for closely related impurities, and so a complete spectrum may not be necessary to obtain a structure. The elucidation of a different substitution pattern, or a 'missing' proton in a particular spin system, may be enough to define a structure, particularly when combined with mass spectrometry data. Also, the use of the related DPFGSE NOE experiment could allow parts of the molecule not in the same spin system to be accessed. Overall, it seems that this method, while not being a panacea for the analysis of all impurities, is nevertheless a useful technique which in this case has provided a fast answer to a real problem.

Notes and references

1 K. Stott, J. Keeler, Q. N. Van and A. J. Shaka, *J. Magn. Reson.*, 1997, **125**, 302.
2 K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang and A. J. Shaka, *J. Am. Chem. Soc.*, 1995, **117**, 4199.
3 L. Braunschweiller and R. R. Ernst, *J. Magn. Reson.*, 1983, **53**, 521.
4 C. Dalvit and G. Bovermann, *Magn. Reson. Chem.*, 1995, **33**, 156.
5 T. Parella, *Magn. Reson. Chem.*, 1996, **34**, 329.
6 A. J. Shaka, C. J. Lee and A. Pines, *J. Magn. Reson.*, 1988, **77**, 274.
7 S. P. Rucker and A. J. Shaka, *Mol. Phys.*, 1989, **68**, 509.
8 A. Davis, G. Estcourt, J. Keeler, E. Laue and J. Titman, *J. Magn. Reson. Ser. A*, 1993, **105**, 167.
9 M. Rance, *J. Magn. Reson.*, 1987, **74**, 557.
10 R. Bazzo and I. D. Campbell, *J. Magn. Reson.*, 1988, **76**, 358.
11 See for example, L. Griffiths, *Anal. Chem.*, 1995, **67**, 22, 4091.
12 H. Barjat, G. A. Morris, S. Smart, A. G. Swanson and S. C. R. Williams, *J. Magn. Reson. Ser. B*, 1995, **108**, 2, 170.
13 D. H. Wu, A. D. Chen and C. S. Johnson, *J. Magn. Reson. Ser. A*, 1996, **121**, 88.

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